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Biochemistry

Sequence of a New World primate insulin having low biological potency and immunoreactivity

(insulin gene/preproinsulin/owl monkey/African green monkey/chimpanzee)

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ABSTRACT **The organization of the insulin gene of the owl or night monkey (*Aotus trivirgatus*), a New World primate, is similar to that of the human gene. The sequences of these two genes and flanking regions possess 84.3% homology. An unusual feature of the owl monkey gene is the partial duplication and insertion of a portion of the A-chain coding sequence into the 3′ untranslated region. The insulin gene of this primate also lacks a region of tandem repeats that is present in the 5′ flanking region of the human and chimpanzee genes. Owl monkey preproinsulin has 85.5% identity with the human insulin precursor and is the most divergent of the primate insulins/preproinsulins yet described. The differences between owl monkey and human preproinsulin include three substitutions in the signal peptide, two in the B chain, seven in the C peptide, and three in the A chain. One of these replacements is the conservative substitution of valine for isoleucine at position A2, an invariant site in all other vertebrate insulins and insulin-like growth factors. The substitutions in owl monkey insulin at B9, B27, A2, A4, and A17 alter its structure so that it has only 20% of the receptor-binding activity and 1% of the affinity with guinea pig anti-porcine insulin antibodies as compared to human insulin.**

The sequences of mammalian insulins are in general highly conserved, differing at only one to four positions and having 92–98% identity (1). Thus, polyclonal antibodies raised against bovine or porcine insulin usually bind insulins of other mammals with only small variations in cross-affinity (2). However, the insulins of some hystricomorph rodents (3) and New World monkeys (4) do not cross-react significantly with these antibodies. The sequences of the insulins from several New World hystricomorphs (guinea pig, coypu, casiragua, cuis, chinchilla) and one Old World species (African porcupine) have revealed that they are, in general, highly divergent, having accumulated 7–22 amino acid replacements and possessing only 57–86% identity with the human hormone (5, 6). These insulins also exhibit other unusual physical and biological properties in addition to their poor cross-reactivity with anti-bovine/porcine insulin serum (cuis and chinchilla insulin can be neutralized by this antisera, indicating that not all hystricomorph rodents possess insulins whose antigenic structure differs from other mammals). They do not self-associate to form dimers or, in the presence of zinc, hexamers (7–10). They also exhibit reduced insulin receptor-binding affinity and low metabolic potency (11) but have enhanced *in vitro* growth-promoting effects (12). The molecular basis for the low cross-reactivity of New World monkey insulins with anti-porcine insulin antibodies has not been examined further since the report of Mann and Crofford in 1970 (4) that only trace amounts of insulin could be detected in the plasma of two New World primates—the capuchin (*Cebus apella*) and squirrel monkey (*Saimiri sciurea*)—whereas an Old World monkey—the rhesus monkey (*Macaca mulatta*)—and the chimpanzee (*Pan troglodytes*) had insulin levels similar to those observed in man. To investigate the structural basis for these differences, we have cloned and sequenced the insulin gene of a New World primate, the owl monkey (*Aotus trivirgatus*), and deduced the amino acid sequence of owl monkey preproinsulin.* In addition, we have purified insulin from the pancreas of this New World primate and determined its biological and immunological properties.

MATERIALS AND METHODS

Insulin Gene Cloning and Sequencing. DNA, isolated from cultured lymphocytes of a female owl monkey [*A. trivirgatus*, karyotype VI ($2n$ = 49/50) (Bolivia)], was a gift from Nancy Shui-Fong Ma. The DNA was digested with *Eco*RI and fragments of 10–15 kilobase pairs (kbp) were isolated by electrophoresis and cloned in λ Charon 4A (13). Phage containing the owl monkey insulin gene were identified by cross-hybridization with the insert from the human insulin gene plasmid phins96 (14).

Subfragments of the 12-kbp owl monkey *Eco*RI fragment containing various parts of the insulin gene were subcloned into M13 mp10 (15) and sequenced by the dideoxy chain-termination method (16). The sequence of the 5′ flanking region of the gene, exons 1–3, and intron A was determined on both strands as was about 75% of the sequence of intron B. The nucleotide sequence was read against and compared with that of the human insulin gene to confirm that differences were not due to sequencing or clerical errors.

Purification and Crystallization of Owl Monkey Insulin. Insulin was purified from three pancreases (1.6 g wet weight) obtained at autopsy. The tissue was homogenized in ice-cold acid/ethanol and the insulin was partially purified from this extract by isoelectric precipitation and gel filtration on a 1.0 × 50 cm Bio-Gel P-30 column (Bio-Rad) with 3 M acetic acid as the eluant (17). Fractions containing insulin, identified by their position of elution, were pooled and concentrated. The insulin in this material was purified to homogeneity by HPLC (18). Zinc insulin crystals were prepared using standard procedures (19).

Insulin and Radioreceptor Assays. Immunoassays were performed as described elsewhere (20) using a guinea pig anti-porcine insulin antibody prepared in this laboratory and anti-human C-peptide antibody M1230. Receptor-binding potency was measured in a displacement assay using IM-9 cells and ^{125}I-labeled human insulin.

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Abbreviation: HVR, hypervariable region.
*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02989).

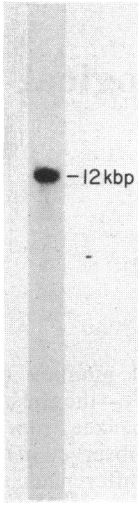

FIG. 1. Southern blot of the owl monkey insulin gene. Five micrograms of DNA was digested with *Eco*RI and after agarose gel electrophoresis was transferred to a nitrocellulose filter that was hybridized with the nick-translated insert from the human insulin plasmid phins96 using standard conditions. The size of the hybridizing fragment is indicated.

RESULTS

The human insulin gene probe hybridizes to a single *Eco*RI fragment of about 12 kbp in a digest of owl monkey DNA (Fig. 1). Ma *et al.* (21) have localized the single owl monkey insulin gene to chromosome 19 (karyotype VI) of this primate. The 12-kbp *Eco*RI fragment was cloned and the sequence of a region of 2113 base pairs (bp) including the 1497-bp insulin gene as well as 510 and 106 bp of 5′ and 3′ flanking sequence, respectively, was determined (Fig. 2). The boundaries of the exons were assigned by comparison with the human insulin gene sequence (22, 23). The overall nucleotide sequence homology between the owl monkey and human sequences is 84.3% (insertions/deletions were counted as one difference regardless of size): upstream of the hypervariable region (HVR), 78.8%; between the HVR and exon 1, 90.7%; exon 1, 75.6%; intron A, 84.9%; exon 2, 91.2%; intron B, 82.6%; exon 3, 82.9%; and 3′ flanking, 79.1%. There is 85.5% identity between the amino acid sequences of owl monkey and human preproinsulin (Fig. 3) and 89.9% nucleotide sequence homology between the protein-coding regions of these two genes.

Owl monkey insulin was purified to homogeneity using a simple three-step procedure starting with 1.6 g of tissue. The final recovery, about 175 μg, is in the range expected. The purified material behaved as a single component on HPLC with a retention time about 3 min longer than human insulin, which is consistent with the differences in the sequences of the owl monkey and human proteins. Owl monkey insulin readily crystallized in the presence of Zn ions at pH 6.1 and, on microscopy, the crystals resembled square-based bipyramids of regular octahedral form, implying 4-3-2 symmetry of a cubic space group (Fig. 4).

The purified owl monkey insulin was only 20% as efficient as human insulin in displacing ^{125}I-labeled human insulin binding to IM-9 cells (Fig. 5). This suggests that the metabolic potency of owl monkey insulin is about 20% that of the human hormone. The owl monkey insulin bound to anti-porcine insulin antibodies about 1% as well as human insulin (Fig. 5). Rat insulin, which consists of approximately equal amounts

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GGCCTAGCTAGGGCTGCTGTCCTGGGGTGGGCTGGGAATGGGCAGCCATCAGGCAGGGGCCCCCTCACTCCCCTACCCCGACAACCTCGGCCCACCCATGGGGGCATCTCGGGCAACCAGA   -390

     (Hypervariable Region)
GATAGAGG GCAGGGGTCTGGGG ACAGCAGCGTGAAGAGCCCCGCCCTGCAGCCTCCCGCACTCCTGGTCTAATGTGGAAAGTGGCCCAGATGAGGGCTTTGCTCTCCTGGAGACATTTGCC -269

CCCAGCTGTGAGCAGGGACAGGACTGGCCACCAGCGCCTGGTTAAGACTCTAATGACCACCCCTCCCGGCCCTGAGGAAGAGGTGCTGACGACCAAGGAGATATTCCCGCAGACCCAGCAG   -148

CCGGGAAATGATCTGGAAAGTGCAGCCTCAGCCCCCAGCCATCTGCCAGCCCCTGCACCTCAGGCCCTAATGGGCCAGGCGGCAAGGTTGGCAGGTAGGGGAGATGGGCTCTGGGCCTATA    -27

                              Exon 1                                  Intron A
AAGCCAGCAGGGACCCAGCAGCCCTC ACGCCCGGGACCAGCTGCATCACAGGAGGCCAGCGAGCAG GTCTGTTCCAAGGGCCTTCGAGCCAGTCTGGGCCCCAGGGCTGCCCCACTCGGGG    95

TTCCAGAGCAGTTGGACCCCAGGTCTCAGCGGGAGGGTGTGGCTGGGCTCTGAAGCATTTGGGTGAGCCCAGGGGCTCAGGGCAGGGCACCTGCCTTCAGCGGCCTCAGCCTGCCTGTCTC    216

                              -24                 -20                                                 -10
     Exon 2                   Met Ala Leu Trp Met His Leu Leu Pro Leu Leu Ala Leu Leu Ala Leu Trp Gly Pro Glu Pro Ala Pro Ala
CCAG GTCTCTGTCCTTCCACC        ATG GCC CTG TGG ATG CAC CTC CTG CCC CTG CTG GCG CTG CTG GCC CTC TGG GGA CCC GAG CCA GCC CCG GCC    309

 1                                       10                                                      20                                                      30
Phe Val Asn Gln His Leu Cys Gly Pro His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Ala Pro Lys Thr
TTT GTG AAC CAG CAC CTG TGC GGC CCC CAC CTG GTG GAA GCC CTC TAC CTG GTG TGC GGG GAG CGA GGT TTC TTC TAC GCA CCC AAG ACC    399

Arg Arg Glu Ala Glu Asp Leu Gln V Intron B
CGC CGG GAG GCG GAG GAC CTG CAG G GTGAGCCCCACCGCCCCTCCGTGCCCCCGCCGCCCCCAGCCACCCCCACTCCCGCTGCTCCCACCCAGCCTGGGCAGAAGGGGACAGG    512

AGGCTGCTACCAGTAGGGAGACAGGTGGACTTTTTAAAAAGAAATGAAGTTCTCTTGGTCACATCCTGAAAGTGACCAGCTCCCTGTGGCCCCGGCAGAATCTCAGCCTGAGGACGGTATT    633

GGCTTCGGCAGCTGAGCTCCGAGATACCTCGGAGGGCACGGCAGGGTAGGGTCCTCCCTCCACGTGCCCCTCGAGCAAATGCCTCGCAGCCCACTTCTCCACCCTCACCTGAGGACCGCAG    754

CTTCCAGTGTTTTGTTGAGTACATCAAGTCCTGGGTGACCTGCGGTCACAGGGTGCCCCACGCTGCCTGCCTGTGGCAAATGCCCCATGGCACCCTGAGGAAGGCATGGCTGCTGCCACGG    875

TGGGCCAGACCCCTGTCCCCGGGCTTCATGACAGCCTCCATAGTCAGGAAATGGGGCAGGCACTGGTGACAGGCCCTGGAAAGACGTACCGGGGGTACCCGTTCAGCCTCCTGCCATGGCA    996

CCACCCAGGGCATTGAAGTCCTGTATGTCCACACCCAGTGTGGGGCACCCTTCCTCAACCTGGGCCCAGCTCGGCTGAGGGGGTGAGGGCGTGACCGGGGCTGGCGGGCAGGCGGGCACC  1,117

                                                                                                     Exon 3
                                                                                                     al Gly Gln Val Glu Leu Gly Gly Gly Ser
ATCTCTCCCTGACTGTGCCATCCTGCGTGCCTCTTCCTCGCCGCTGTTCCGGACCTGCTCTGCGTGGCTCGCCCTGGCAG                      TG GGG CAG GTG GAG CTG GGT GGG GGC TCT  1,226

    50                                      60                                         70
Ile Thr Gly Ser Leu Pro Pro Leu Glu Gly Pro Met Gln Lys Arg Gly Val Val Asp Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln
ATC ACG GGC AGC CTG CCA CCC TTG GAG GGT CCC ATG CAG AAG CGT GGC GTC GTG GAT CAG TGC TGC ACC AGC ATC TGC TCC CTC TAC CAG  1,316

    80                 84
Leu Gln Asn Tyr Cys Asn AM
CTG CAG AAC TAC TGC AAC TAG|ACTGGGCCCACAGCGACCCCGTGCCCACTGCCACCTGCACCAGCACCTGCTCCCTCTACCAGCTGGAGAACCACAGCTGGCTGCGGCCC|ACAG 1,430

                                                              Polyadenylation Site
CAGGCCCAAATGCGGCCCTGCACCCTCCTCACCTGCACATGAGTGATGGAATAAAGCCTTGAACCAGC TCTGCTGTGCTGTTTGCGTGTTTGGGGGGCCCTGGGCAGACCCCGCCGTCCTG 1,551

GCACTGTTATGAGCCCCTCCCAGCTCTCTCCAAGCTCTCGCCCGGCCTGCAG                                                                  1,603
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FIG. 2. Sequence of the owl monkey insulin gene and flanking regions. The predicted start of transcription of the gene has been designated as nucleotide 1. The deduced amino acid sequence of owl monkey preproinsulin is indicated. The signal peptide is amino acids −24 to −1; the B chain, 1–30; C peptide (including the pairs of adjacent basic residues), 31–63; and A chain, 64–84. The location of the HVR in the 5′ flanking region of the human insulin is noted. The owl monkey gene lacks a similar region. The number of the nucleotide at the end of each line is shown. The boundaries of the 89-bp insertion in the 3′ untranslated region are indicated by vertical lines and the imperfect 43-bp duplication is underlined.

Signal Peptide

	-24				-20										-10									-1
HUMAN	Met	Ala	Leu	Trp	Met	Arg	Leu	Leu	Pro	Leu	Leu	Ala	Leu	Leu	Ala	Leu	Trp	Gly	Pro	Asp	Pro	Ala	Ala	Ala
CHIMP	—	—	—	—	—	—	—	—	—	—	—	Val	—	—	—	—	—	—	—	—	—	—	Ser	—
MACAQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Pro	—
AFGM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Val	Pro	—
OWLM	—	—	—	—	—	His	—	—	—	—	—	—	—	—	—	—	—	—	—	Glu	—	—	Pro	—
GP	—	—	—	—	—	His	—	—	Thr	Val	—	—	—	—	—	—	—	—	—	Asn	Thr	Gly	Gln	—

B-Chain

	B1									B10										B20										B30
HUMAN	Phe	Val	Asn	Gln	His	Leu	Cys	Gly	Ser	His	Leu	Val	Glu	Ala	Leu	Tyr	Leu	Val	Cys	Gly	Glu	Arg	Gly	Phe	Phe	Tyr	Thr	Pro	Lys	Thr
CHIMP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MACAQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AFGM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
OWLM	—	—	—	—	—	—	—	—	Pro	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Ala	—	—	—
GP	—	—	Ser	Arg	—	—	—	—	—	Asn	—	—	—	Thr	—	—	Ser	—	—	Gln	Asp	Asp	—	—	—	—	Ile	—	—	Asp

C-Peptide

			C1									C10										C20										C31		
HUMAN	Arg	Arg	Glu	Ala	Glu	Asp	Leu	Gln	Val	Gly	Gln	Val	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Ser	Leu	Gln	Pro	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys	Arg
CHIMP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MACAQ	—	—	—	—	—	—	Pro	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AFGM	—	—	—	—	—	—	Pro	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
OWLM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Ser	Ile	Thr	—	—	—	Pro	—	—	|||	|||	—	—	Pro	Met	—	—	—
GP	—	—	—	Leu	—	—	Pro	—	—	Glu	—	Thr	—	—	—	Met	—	Leu	—	—	—	Gly	—	—	—	—	—	—	—	Met	Ala	—	—	—	—

A-Chain

	A1									A10											A21
HUMAN	Gly	Ile	Val	Glu	Gln	Cys	Cys	Thr	Ser	Ile	Cys	Ser	Leu	Tyr	Gln	Leu	Glu	Asn	Tyr	Cys	Asn
CHIMP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
MACAQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
AFGM	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
OWLM	—	Val	—	Asp	—	—	—	—	—	—	—	—	—	—	—	—	Gln	—	—	—	—
GP	—	—	—	Asp	—	—	—	—	Gly	Thr	—	Thr	Arg	His	—	—	Gln	Ser	—	—	—

FIG. 3. Comparison of primate and guinea pig preproinsulin sequences. Only differences from the human sequence are indicated: HUMAN, human; CHIMP, chimpanzee; MACAQ, cynomolgus monkey; AFGM, African green monkey; OWLM, owl monkey; and GP, guinea pig. The cynomolgus and African green monkey are Old World monkeys and are representatives of two difference genera of the subfamily Cercopithecinae. The triplets of short vertical lines in the C peptide of the owl monkey sequence indicate the deletion of two amino acids in this region. The cynomolgus monkey insulin sequence is from Wetekam *et al.* (24) and the guinea pig sequence is from Chan *et al.* (5) and Watt (25). The human, chimpanzee, African green monkey, and owl monkey sequences are from Bell *et al.* (22), this publication, and our unpublished observations.

of two nonallelic molecules of slightly different sequence (1), cross-reacted with these antibodies about 50% as well as human insulin.

We also attempted to measure insulin and C peptide in the sera of three owl monkeys before and after a meal using standard clinical radioimmunoassay procedures. However we were unable to detect either insulin or C peptide.

DISCUSSION

The organization of the owl monkey insulin gene is similar to that of the human insulin gene (22, 23). However, there are

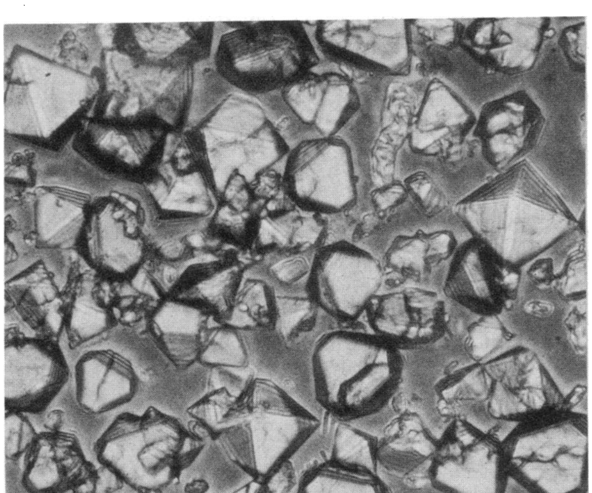

FIG. 4. Crystals of owl monkey insulin. (×250.)

notable differences. First, the 5′ flanking region of the owl monkey sequence lacks a region of tandem repeats analogous to the HVR of the human gene (26) and instead has a sequence—GCAGGGGTCTGGGG—that is closely related to the consensus repeat—ACAGGGGTGTGGGGG—of the human HVR. The insulin gene of an Old World monkey, the African green monkey (*Cercopithecus aethiops*) (S.S. and G.I.B., unpublished data) also lacks a HVR-like region and has in its place a sequence, ACAGGGTCCCAGG ACAG-GGGGTCTGGGG, that is homologous to two copies of the consensus repeat found in the human insulin gene HVR. There is a region of 21 repeats of the consensus sequence ACAGGGGTCCTGGGG flanking the chimpanzee insulin gene (S.S. and G.I.B., unpublished data). Thus, this region of tandem repeats, which in the human constitutes the polymorphic HVR (14, 26), appears to have arisen during evolution of the higher apes. The reasons for the tandem duplication of a sequence in this region are unknown. Second, the part of the owl monkey gene encoding the 3′ untranslated region of the mRNA is much longer than the cognate region of the human gene due to the insertion of an 89-bp segment following the TAG stop codon (Fig. 2). This 89-bp "insert" contains a 43-bp segment (underlined in Fig. 2) that is an imperfect direct duplication—there are four mismatches—of part of the region encoding the A chain of owl monkey insulin. Similar partial duplications of the protein-coding region of the insulin gene have not been observed in other vertebrates. The extent to which this 43-bp duplication or even duplications of other regions of the owl monkey gene, which were subsequently lost, facilitated the accumulation of the large number of amino acid replacements described below is unknown.

The predicted amino acid sequence of owl monkey insulin (Figs. 2 and 3) is not identical to any vertebrate or primate

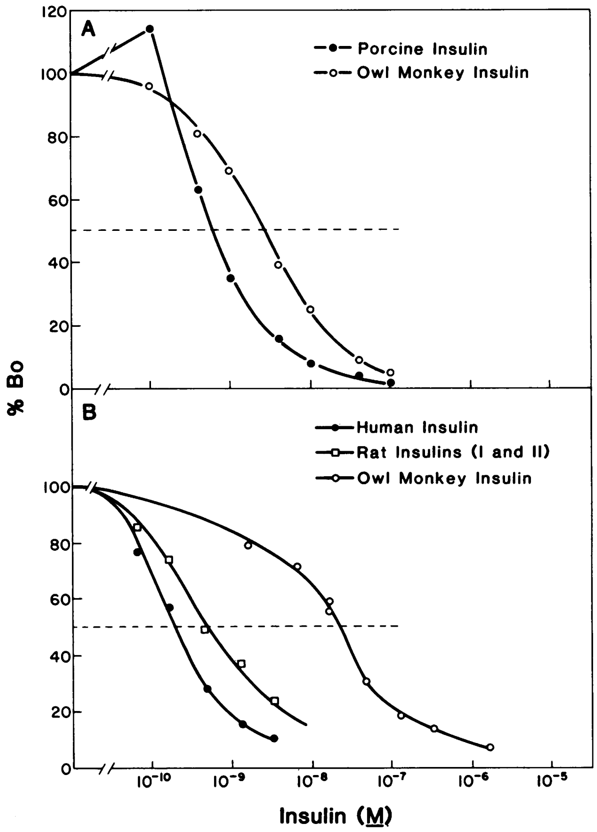

FIG. 5. Properties of owl monkey insulin. % Bo, % bound. (*A*) Receptor binding. Displacement of human insulin ^{125}I-labeled at tyrosine-14 of the A chain from IM-9 cells by owl monkey and porcine insulin. (*B*) Antibody binding. Inhibition of binding of human insulin ^{125}I-labeled at tyrosine-14 of the A chain to guinea pig anti-porcine antibodies by owl monkey, rat, and human insulin.

insulin yet characterized (1). Its sequence differs at five positions from that of human insulin having substitutions at residues B9, B27, A2, A4, and A17. By contrast, the sequences of insulins from the chimpanzee and two Old World monkeys, the cynomolgus monkey (*Macaca fascicularis*) (24) and African green monkey, are identical to human (Fig. 3). The sequence of owl monkey preproinsulin is thus the most divergent of the known primate sequences. The COOH-terminal half of the C peptide is particularly different having six replacements and a deletion of two residues.

Of the amino acid replacements occurring in owl monkey insulin, the conservative substitution of valine for isoleucine at A2 is particularly interesting as this is an invariant site in all insulins (1) as well as insulin-like growth factors I and II (6, 27, 28). Of the other changes, B9 proline occurs in rat I and mouse I insulins, and A4 aspartic acid and A17 glutamine are present separately or together in a large number of insulins. Residue B27 is a highly variable site in the insulin B chain; however, alanine has not been previously reported at this position. Two of the five amino acid substitutions in owl monkey insulin (A4 aspartic acid and A17 glutamine) also occur in the guinea pig sequence (Fig. 3). A third (B27) is a hydrophobic amino acid in both owl monkey and guinea pig insulin and an uncharged polar or basic residue in all other mammalian insulins.

Comparison of the biological and immunological properties of owl monkey and human insulins indicates that owl monkey insulin has only 20% of the potency and 1% of the immunoreactivity of human insulin. Computer-graphics model building of the owl monkey insulin sequence, kindly carried out by T. L. Blundell and his colleagues (Birkbeck College, University of London), based on the high-resolution porcine insulin structure (29) suggested that the amino acid replacements in the owl monkey sequence, taken together, would be expected to have only minor effects upon the tertiary and quarternary structure of the molecule. The A17 glutamine substitution could possibly reduce hexamer stability and account in part for the low reactivity of owl monkey insulin with anti-porcine insulin antibodies. The side chains of residues B9, B27, A4, and A17 are near the periphery of the receptor-binding region and substitutions at these positions might have a marginal effect on receptor binding. It is likely that the isoleucine → valine substitution A2 is responsible for the reduced potency of owl monkey insulin. This site, invariant in all other vertebrate insulins, presumably fulfills an important structural function in stabilizing the hydrophobic core of the insulin molecule and is required for insulin's full biological potency. The studies carried out by Katsoyannis and colleagues (30, 31) on A2 norleucine, leucine and glycine analogues of sheep insulin, support this contention. These analogues exist as monomers under conditions (12–14 μM) where normal insulin is predominantly a dimer and have receptor-binding affinities and potencies of <1% of normal insulin. They are weak but full agonists, reaching the same maximal activity as insulin but at much greater concentrations. In contrast to these analogues, the A2 substitution in owl monkey insulin is relatively more conservative. This probably accounts for the preservation of some biological activity of owl monkey insulin compared to the other A2 analogues.

The owl monkey and the capuchin and squirrel monkey, which were studied by Mann and Crofford (4), belong to different subfamilies of New World primates, Aotinae and Cebinae, respectively. The low immunoreactivity of insulin from all three species with anti-porcine insulin antibodies suggests that this will be a property of the insulins of primates belonging to the family Cebidae. Moreover, it is likely that the insulins of other New World primates will also be relatively highly substituted.

The common continental isolation of the New World hystricomorphs and primates suggests that a similar positively acting selective factor might have caused the high rate of divergence of insulin observed in these mammals. It has been suggested that local shortages of zinc (32) or the requirement for an insulin molecule with increased relative growth-promoting activity (9, 12) may have been responsible factors. However, unlike guinea pig, owl monkey insulin is not substituted at B10 and has apparently normal zinc-binding properties. For this and other reasons discussed elsewhere in more detail (5, 33), zinc deficiency is unlikely to have contributed to the increased mutation rate in the insulins of these New World species. An alternative explanation could be that the divergent evolution of insulin in these two mammalian groups has been in response to a common environmental agent such as an insulin-requiring parasite(s). Thus, just as *Plasmodium falciparum* synthesizes a transferrin receptor (34), some parasites may synthesize an insulin receptor-like molecule that serves to increase nutrient uptake and stimulate metabolic activity in the parasite or in parasite-infected cells. One evasive strategy against such parasites might include alterations in insulin structure such that it becomes either a less efficient agonist or even an antagonist for the parasite-encoded receptor.

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1. Hallden, G., Gafvelin, G., Mutt, V. & Jornvall, H. (1986) *Arch. Biochem. Biophys.* **247**, 20–27.
2. Pope, C. G. (1986) *Adv. Immunol.* **5**, 209–244.
3. Neville, R. W. J., Weir, B. J. & Lazarus, N. R. (1973) *Diabetes* **22**, 851–853.
4. Mann, G. V. & Crofford, O. B. (1970) *Science* **169**, 1312–1313.
5. Chan, S. J., Episkopou, V., Zeitlin, S., Karathanasis, S. K., MacKrell, A., Steiner, D. F. & Efstratiadis, A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5046–5050.
6. Dafgard, E., Bajaj, M., Honegger, A., Pitts, J., Wood, S. & Blundell, T. (1985) *J. Cell Sci.*, Suppl. **3**, 53–64.
7. Zimmerman, A. E., Wells, D. I. C. & Yip, C. C. (1972) *Biochem. Biophys. Res. Commun.* **46**, 2127–2133.
8. Wood, S. P., Blundell, T. L., Wollmer, A., Lazarus, N. R. & Neville, R. W. J. (1975) *Eur. J. Biochem.* **55**, 531–542.
9. Horuk, R., Blundell, T. L., Lazarus, N. R., Neville, R. W. J., Stone, D. & Wollmer, A. (1980) *Nature (London)* **286**, 822–824.
10. Bajaj, M., Blundell, T. L., Horuk, R., Pitts, J. E., Wood, S. P., Gowan, L. K., Schwabe, C., Wollmer, A., Gliemann, J. & Gammeltoft, S. (1986) *Eur. J. Biochem.* **238**, 345–351.
11. Horuk, R., Goodwin, P., O'Conner, K., Neville, R. W. J., Lazarus, N. R. & Stone, D. (1979) *Nature (London)* **279**, 439–440.
12. King, G. L. & Kahn, C. R. (1981) *Nature (London)* **292**, 644–646.
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
14. Bell, G. I., Horita, S. & Karam, J. H. (1984) *Diabetes* **33**, 176–183.
15. Messing, J. (1983) *Methods Enzymol.* **101**, 20–78.
16. Sanchez-Pescador, R. & Urdea, M. S. (1984) *DNA* **3**, 339–343.
17. Steiner, D. F., Cho, S., Oyer, P. E., Terris, S., Peterson, J. D. & Rubenstein, A. H. (1971) *J. Biol. Chem.* **246**, 1365–1374.
18. Rivier, J. & McClintock, R. (1983) *J. Chromatogr.* **268**, 112–119.
19. Schlichtkrull, J. (1956) *Acta Chem. Scand.* **10**, 1455–1458.
20. Madsen, O. D., Frank, B. H. & Steiner, D. F. (1984) *Diabetes* **33**, 1012–1016.
21. Ma, N. S. F., Gerhard, D. S., Housman, D. E., Orkin, S. & Bruns, G. (1986) *Cytogenet. Cell Genet.* **43**, 57–68.
22. Bell, G. I., Pictet, R. L., Rutter, W. J., Cordell, B., Tischer, E. & Goodman, H. M. (1980) *Nature (London)* **284**, 26–32.
23. Ullrich, A., Dull, T. J., Gray, A., Brosius, J. & Sures, I. (1980) *Science* **209**, 612–615.
24. Wetekam, W., Groneberg, J., Leineweber, M., Wengenmayer, F. & Winnacker, E.-L. (1982) *Gene* **19**, 179–183.
25. Watt, V. M. (1985) *J. Biol. Chem.* **260**, 10926–10929.
26. Bell, G. I., Selby, M. & Rutter, W. J. (1982) *Nature (London)* **295**, 31–35.
27. Bell, G. I., Stempien, M. M., Fong, N. M. & Rall, L. B. (1986) *Nucleic Acids Res.* **14**, 7873–7882.
28. Stempien, M. M., Fong, N. M., Rall, L. B. & Bell, G. I. (1986) *DNA* **5**, 357–361.
29. Dodson, G. G., Dodson, E. J., Reynolds, C. D. & Vallely, D. C. (1980) in *Insulin: Chemistry, Structure and Function of Insulin and Related Hormones*, eds. Brandenburg, D. & Wollmer, A. (de Gruyter, Berlin), pp. 9–16.
30. Okada, Y., Chanley, J. D., Burke, G. T. & Katsoyannis, P. G. (1981) *Hoppe-Seyler's Z. Physiol. Chem.* **362**, 629–638.
31. Kitagawa, K., Ogawa, H., Burke, G. T., Chanley, J. D. & Katsoyannis, P. G. (1984) *Biochemistry* **23**, 1405–1413.
32. Blundell, T. L. & Wood, S. P. (1975) *Nature (London)* **257**, 197–203.
33. Seino, S., Blackstone, C. D., Chan, S. J., Whittaker, J., Bell, G. I. & Steiner, D. F. (1987) *Horm. Metab. Res.*, in press.
34. Rodriguez, M. H. & Jungery, M. (1986) *Nature (London)* **324**, 388–391.